Alexandria Journal of Veterinary Sciences
www.alexjvs.com

AJVS. Vol. 59 (1): 119-124. Oct. 2018
DOI: 10.5455/ajvs.10556

Polymorphism of IGF-1 and Its Receptor (IGF-1R) Genes and Their Association with Fertility Problems of Egyptian Buffaloes

Sherif I. Ramadan[1]*, Zienab H. Abo-Gamil[1], Hadeel S. El-Qaliouby[1, 2]
[1]Department of Animal Wealth Development, Faculty of Veterinary Medicine, Benha University, Egypt.
[2]Key Laboratory of Animal Genetics, Breeding and Reproduction, Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, 430070, People's Republic of China

Key words:
Egyptian buffalo; Anestrus; polymorphism; IGF-1; IGF-1R

***Correspondence to:**

Sherif.ramadan@fvtm.bu.edu.eg

ABSTRACT

The objectives of our study were to detect genetic polymorphisms in insulin-like growth factor-1 (IGF-1) and insulin-like growth factor1- receptor (IGF-1R) genes by using SCCP and direct sequence methods and to investigate their possible associations with fertility problems in Egyptian buffaloes. A total number of 100 buffalo heifers at Mahallet Mousa farm in Kafr El-sheikh province, Egypt were investigated. Animals were assigned into two main groups: normal fertile (50 animals) and infertile due to anestrous (50 animals). Blood samples were collected and the genomic DNA was extracted then PCR were performed with annealing temperatures at 58ºC and 55ºC for IGF1 and IGF1-R respectively. SSCP was performed for all amplified samples and loaded into a non-denaturing 12% polyacrylamide gel. Four amplicon from both of IGF-1 and IGF-1R genes for the two investigated groups were sequenced using the same primers. 265-bp from 5-untranslated region (5-UTR) of IGF-1 gene and 311-bp from IGF-1R (part of intron 12 and part of exon 13) were amplified. In all investigated samples there was one SSCP pattern for IGF-1 and for IGF-1R genes in anestrus and normal fertile buffaloes' groups Moreover there were no polymorphisms neither in IGF-1 nor IGF-1R after the sequencing of four samples from each buffaloes' group for both of IGF-1 and IGF-1R genes. It seems that IGF-1 and IGF-1R are highly conserved in the investigated buffalo's population.

1. INTRODUCTION

Water buffaloes are the most important domestic animal in Egypt; they are the main source of milk where they contribute for approximately 70% of the total milk production in Egypt. Moreover, buffalo's milk is preferred by the Egyptians because of its white color, high fat content and good flavor (El-Salam and El-Shibiny, 2011). The total numbers of buffaloes in Egypt are about 4,164,928 head, with 2,650,000 ton of milk production (Hassan et al., 2017). However, buffaloes have a lower reproductive performance than cattle. This lower performance may be attributed to late maturity, long postpartum anestrus interval, poor conception rate and seasonal reproductive activity. Anestrus due to ovarian dysfunction is the major reproductive disorders in Egyptian buffaloes (Sosa et al., 2016). However, little efforts have been made to improve their genetic potentiality for productive and reproductive traits (Ibrahim, 2012)

Reproductive performance is one of the most determinant factors for dairy farm profitability. Changes of the insulin-like growth factor-1 (IGF-1) concentration after calving are important metabolic factor affecting the postpartum ovarian activity and eventually the animal's reproductive performance (Kadivar et al., 2012). It is an endocrine hormone and has been strongly associated with several

reproductive and productive characters in dairy animals. IGF-1 hormone in blood has been suggested as potential physiological marker for improved breeding efficiency and profitability in dairy cattle production. It plays a key role in the reproduction because it has a positive effect on cell proliferation, transformation and differentiation (Kumar and Laxmi, 2015). Low concentration of IGF-1 in circulation of postpartum dairy cattle has led to anestrous condition (Anand and Sehgal, 2014). It can affect reproductive performance through its effect on neural pathway controlling the production of gonadotropin-releasing hormone (GnRH). It also has a direct effect on the ovary and its sensitivity to FSH and LH hormones (Lucy, 2000). Polymorphisms within the IGF-I gene and its receptor (IGF-IR) affect the reproductive parameters in dairy animals (Ahmed et al., 2011, Mostafa, 2011, Kadivar et al., 2012, Ararouti et al., 2013, Nicolini et al., 2013).

Genetic variant has been used by the animal breeders as a selection tool for selection of the best animal. Single-strand conformation polymorphism (SSCP) is considered as a powerful technique for detection of sequence variation in the amplified DNA fragments. Searching for SSCP polymorphisms might lead to the finding of genetic markers which could be used as a tool for selection of traits of economic importance (Zhou et al., 2015). Studies on the association of IGF-1 genetic variations with reproductive performance of Egyptian buffaloes still few. The aims of this study were to identify genetic polymorphisms in insulin-like growth factor-1 (IGF-1) and insulin-like growth factor1- receptor (IGF-1R) genes by using SCCP and direct sequence methods and to investigate their possible associations with fertility problems in Egyptian buffaloes.

2. MATERIALS AND METHODS:

All of the experimental procedures were performed in the Central Laboratory of Faculty of Veterinary Medicine, Benha University, Egypt, and were approved by the Animal Ethical Committees of the Benha University.

2.1. Animals and sample preparation: This study was performed on a total number of 100 buffalo heifers at Mahallet Mousa farm in Kafr El-sheikh province, Egypt. Heifers were naturally served for the first time when they reach 300 to 350 kg of body weight and/or 24 months of age. Animals were assigned into two main groups: normal fertile (50 animals) and infertile due to anestrous (50 animals). A total of 50 apparently healthy buffaloes matched for age and body weight, showing normal estrus signs and kept under approximately the same managemental conditions were included as normal fertile and heifers with small and smooth ovaries (no palpable follicles) and lack of estrous signs were diagnosed to be infertile. Buffaloes were diagnosed infertile by the aid of rectal palpation and ultrasonographic examination once a week for at least four successive weeks

2.2. DNA extraction and amplification:

Blood samples were collected from the jugular veins of 50 animals in each group into sterilized vacutainer tubes containing EDTA. The genomic DNA was extracted from the leucocytes using Gene JET genomic DNA extraction kits following the manufacturer protocol (Thermo Scientific). The IGF1 locus was amplified by using PCR forward primer5'-ATTACAAAGCT-GCCTGCCCC-3' and reverse primer CACATCT-GCTAATACACCTTACCCG-3' (Ge et al., 1997) while IGF1-R using forward primer 5'-ACCCGCC-AAGAAATTGTTTC-3'and reverse primer 5'-GGC-TCCTCCATACTTCCTG-TA-3' (Schoenau et al., 2005). The PCR reactions were performed with a total volume 25µl containing 1.0µl of forward and reverse primers (10 pmol), 3.0µl DNA template, 12.5µlMaster mix and 7.5µl nuclease free water. PCR reaction were as follows: 2 min at 94ºC for initial denaturation, 35 cycles of amplification (2 min at 94ºC for denaturation, 1 min at 58ºC and 55ºC of annealing for IGF1 and IGF1-R respectively, 2min at 72ºC for extension) and final extension at 72ºC for 10 min. The size and quality of PCR amplicons were checked on 1.5 % agarose gels.

2.3. Mutation identification by single-strand conformation polymorphisms (SSCP) and direct sequence methods: SSCP was performed for all amplified DNA samples as previously described by El-Magd et al. (2014). 5µl of the PCR product was mixed with 5µl of denaturing solution (95% formamide, 25 mM EDTA, 0.025% bromophenol blue and 0.025% xylene-cyanole) heated at 94ºC for 5 min then suddenly cooled on ice and loaded into a non-denaturing 12% polyacrylamide gel (39:1 acrylamide to bis-acrylamide). The SSCP gel was run in 1 Tris borate EDTA (TBE) buffer at 200 V for 10 h at 4ºC. 0.1% silver nitrate was used for the detection of DNA bands in the gel. Sixteen PCR products (four

amplicon from both of IGF-1 and IGF-1R genes for the two investigated groups) were purified using a PCR purification kits (Roche, Mannheim, Germany), then sequenced. We used the same primers with Big Dye Terminator for sequencing procedure according to the standard protocol (Applied Biosystems, Foster City, CA, USA). We electrophoresed the resultant product on an ABI PRISM 3500xl sequencer (Applied Biosystems, Foster City, CA, USA). We used BLAST software (Boratyn et al., 2012) for identification of sequences. Finch TV 1.4.0 (Geospiza, Inc., Seattle, WA, USA; http://www.geospiza.com) and MEGA 7 (Kumar et al., 2016) softwares were used for polymorphism detection and sequence alignment.

3. RESULTS AND DISCUSSIONS

We successfully amplified 265-bp from 5-untranslated region (5-UTR) of IGF-1 gene and 311-bp from IGF-1R (part of intron 12 and part of exon 13) as shown in figures 1 & 2. PCR-SSCP of the amplified IGF-1 and IGF-1R fragments was performed to detect any polymorphism that might be present. In all investigated samples there was one SCCP pattern for IGF-1 and for IGF-1R genes in anestrus and normal fertile buffaloes' groups as shown in figures 3 & 4. For confirmation of the obtained results we sequenced four samples from each buffaloes' group for both of IGF-1 and IGF-1R genes. There were no polymorphisms neither in IGF-1 nor IGF-1R as shown in figures 5 & 6.

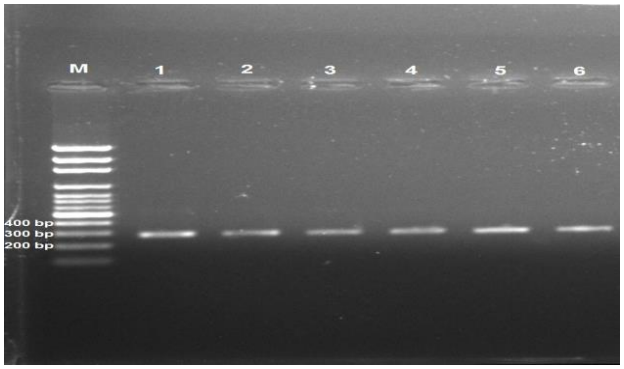

Fig 1. Ethidium bromide stained agarose gel showing the PCR product of buffalo IGF-1gene in Egyptian water buffaloes. M: 100-bp ladder. Lanes 1-6: 265-bp PCR product of IGF-1gene.

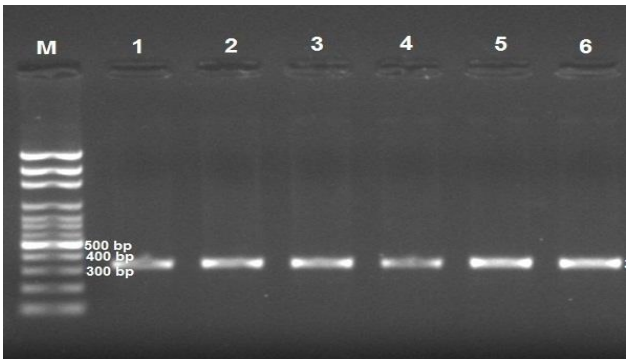

Fig 2. Ethidium bromide stained agarose gel showing the PCR product of IGF-1R gene in Egyptian water buffaloes M: 100-bp ladder. Lanes 1-6: 311-bp PCR product of IGF-1R gene.

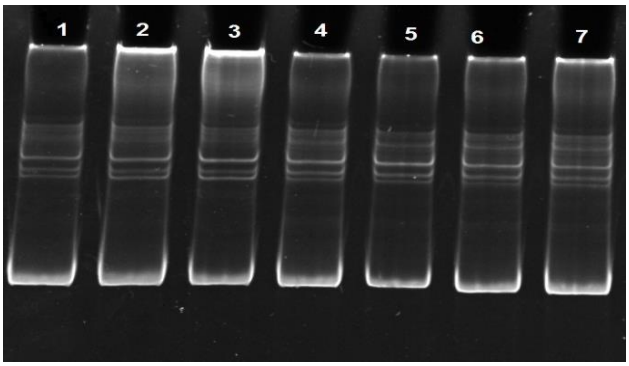

Fig 3. PCR-SSCP patterns of IGF-1 gene showing one SSCP band pattern in Egyptian water buffaloes.

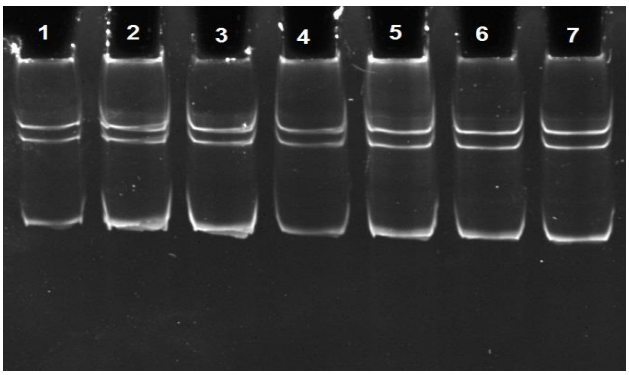

Fig 4. PCR-SSCP patterns of IGF-1R gene showing one SSCP band pattern in Egyptian water buffaloes.

It seems that IGF-1 and IGF-1R are highly conserved in the investigated buffalo's population. This might be attributed to high inbreeding coefficient in this farm or there was some kind of relationship among the sampled animals. Moreover, these two genes (IGF-1 and IGF-1R) might not be exposed to any kind of selection.

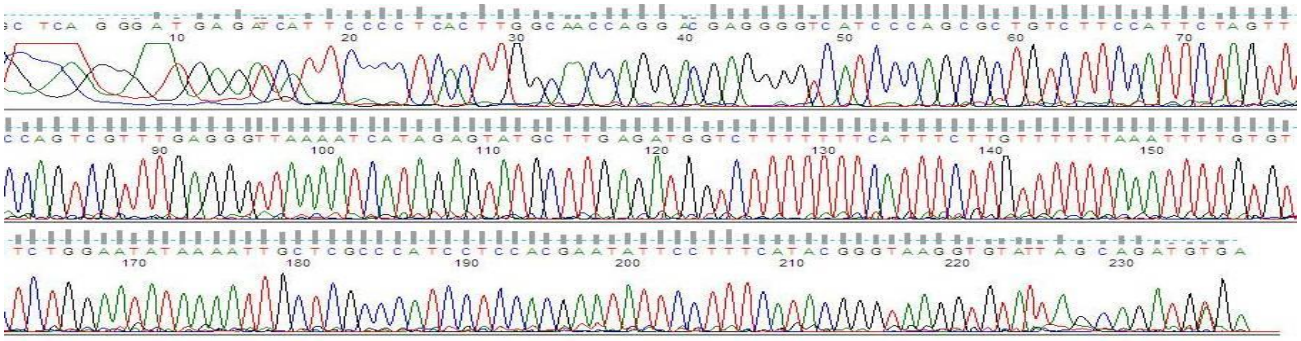

Fig 5. Sequence chromatogram of part of 5-UTR of IGF-1 (265-bp) in Egyptian water buffaloes.

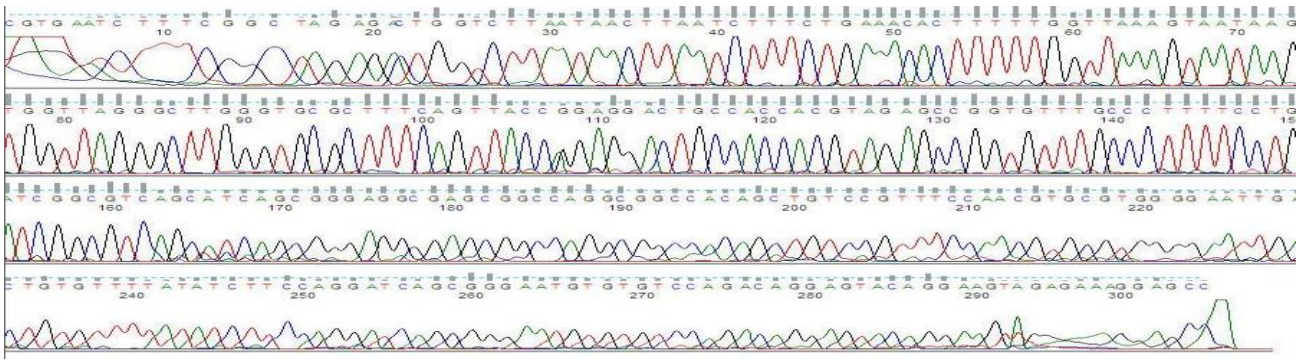

Fig 6. Sequence chromatogram of parts of intron 12 and exon 13 of IGF-1R (311-bp) in Egyptian water buffaloes.

Similar results were obtained by Othman et al. (2013); they found no polymorphism within 250-bp DNA fragment of the regulatory region of the IGF-1 gene and a 616-bp DNA fragment of the IGF-1R gene of Egyptian buffaloes by applying *SnaBI* and *TaqI* restriction endonuclease on IGF-1 and IGF-1R respectively. Moreover, our results were consistent with Fatima et al. (2009), although they observed three types of SSCP band patterns of IGF-1 gene across three Indian buffalo breeds (Mehsani, Surti and Jaffarabadi buffaloes), they found no polymorphism in this gene across the same buffaloes' samples by applying DNA direct sequencing methods and attributed these contradicted results to the independence of SSCP techniques from DNA direct sequence method. On contrary, Khederzadeh and Yazdanpanah (2013) observed three different SSCP patterns in amplified 265-bp of IGF-1 promoter region collected from five cities in Khuzestan province of Iran. Moreover, two novel single nucleotide polymorphisms (G64A and G280A) in the non-coding sequences of IGF-1 exon 1 and exon 4 of Egyptian buffaloes were detected by El-Magd et al. (2017).

Reproduction and growth are the most important two traits of the dairy animals to be considered for genetic improvement (Singh et al., 2014). For a profitable dairy farming the buffaloes are expected to yield a calf almost every year. To achieve that, the dam should resume the estrous cyclicity as soon as possible after calving. Though post-partum anestrus is common in buffaloes, it becomes a serious problem that affects buffalo's fertility and productivity (Kumar and Laxmi, 2015). Functional mutations of causal genes or the genetic markers that are in linkage with the causal genes could be used as tools for selecting animals for breeding programs. Selection of the best genotypes for dairy animal's reproduction is of pivotal importance to maximize farm profitability (Singh et al., 2014). IGF-1 plays a key role in animal's reproduction because of its positive effect on cell proliferation, transformation and differentiation (Kumar and Laxmi, 2015). Although the importance of IGF-1 and its receptors on reproduction and fertility, we didn't find any polymorphism between the anestrus and normal fertile buffaloes' groups for the studied two loci. We recommend increasing the numbers of the investigated animals and searching

for other loci within these two important genes (IGF-1 and IGF-1R).

4. CONFLICT OF INTERESTS

The authors declared that they have no conflict of interest

5. ACKNOWLEDGMENT

The authors wish to thank Dr. Samy Darwish the director of buffalo's farm at Mahallet Mousa, Kafr El-Sheikh Province, Egypt, for allowing them to collect the blood samples and reproductive data.

6. AUTHOR CONTRIBUTION

SR planned and designed the study. ZG collected the blood samples and perform the laboratory experiments. SR and HE performed the statistical analysis and interpretation of laboratory results. SR wrote the first draft of the manuscript. All authors contributed in the revision and approved the final manuscript.

7. REFERENCES

Ahmed, S. S., Abd el Aziz, K. B., Hassan, N. A., Mabrouk, D. M. 2011. Genetic polymorphism of some genes related to reproductive traits and their association with calving interval in Egyptian buffalo. G. Q. G. 3: 13-19.

Anand, L. N., Sehgal, J. P. 2014. Relationship between plasma IGF I, body weight and age at puberty in low body weight murrah calves and effect of supplementation of fermented yeast culture in the improvement of productive parameters. I. J. A. B. R. 4: 369-373.

Ararouti, T., Mirzael, A., Sharifiyazdi, H. 2013. Assessment of single nucleotide polymorphism in the 5'-flanking region of Insulin-like growth factor-I (IGF-I) gene as a potential genetic marker for fertility in Holstein dairy cows. stanbul Üniv. Vet. Fak. Derg. 39: 175-182.

Arefaine, H., Kashwa, M. 2015. A review on strategies for sustainable buffalo milk production in Egypt. J. Biol. Agric. Healthc. 5: 63-67.

Boratyn, G. M., Schäffer, A. A., Agarwala, R., Altschul, S. F., Lipman, D. J., Madden, T. L. 2012. Domain enhanced lookup time accelerated BLAST. Biol. Direct. 7: 12.

El-Magd, M. A., Abo-Al-Ela, H. G., El-Nahas, A., Saleh, A. A., Mansour, A. A. 2014. Effects of a novel SNP of IGF2R gene on growth traits and expression rate of IGF2R and IGF2 genes in gluteus medius muscle of Egyptian buffalo. Gene. 540: 133-139.

El-Magd, M. A., Saleh, A. A., Nafeaa, A. A., El-Komy, S. M., Afifi, M. A. 2017. Polymorphisms of the IGF1 gene and their association with growth traits, serum concentration and expression rate of IGF1 and IGF1R in buffalo. J. Zhejiang Univ. Sci. B. 18: 1064-1074.

El-Salam, M. H. A., El-Shibiny, S. 2011. A comprehensive review on the composition and properties of buffalo milk. Dairy Sci. Technol. 91: 663.

Fatima, S., Bhatt, S., Bhong, C., Rank, D., Joshi, C. 2009. Genetic polymorphism study of IGF-I gene in buffaloes of Gujarat. Buffalo Bull. 28: 159-164.

Ge, W., Davis, M., Hines, H. 1997. Two SSCP alleles detected in the 5'-flanking region of bovine IGF1 gene. Anim. Genet. 28: 155-156.

Hassan, F. A., Ali, M. A., El-Tarabany, M. S. 2017. Economic impacts of calving season and parity on reproduction and production traits of buffaloes in the sub-tropics. Environ. Sci. Pollut. Res. 24: 10258-10266.

Ibrahim, M. A. R. 2012. Water buffalo for our next generation in Egypt and in the world. Scientific Papers Anim. Sci. . 55: 183-192.

Kadivar, A., Ahmadi, M. R., Gheisari, H. R., Nazifi, S. 2012. Assessment of IGF-I as a factor influencing postpartum reproductive performance and ovarian condition in dairy cattle. Comp. Clin. Path. 21: 589-596.

Khederzadeh, S., Yazdanpanah, A. 2013. Polymorphisms in the 5´ flanking region and first exon of insulin like growth factor 1 gene in southern populations of buffalo. Am. J. Biochem. Biotechnol. 9: 348-354.

Kumar, A., Laxmi, N. A. 2015. Role of IGF 1 in male and female reproduction in bovines: a review. Asia. Pac. j. res. 1: 17-25.

Kumar, S., Stecher, G., Tamura, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33: 1870-1874.

Lucy, M. 2000. Regulation of ovarian follicular growth by somatotropin and insulin-like growth factors in Cattle1. J. Dairy Sci. 83: 1635-1647.

Mostafa, D. 2011. Genetic polymorphism of some genes related to reproduction in the river buffalo. MS Thesis, Faculty of Science, Ain Shams University.

Nicolini, P., Carriquiry, M., Meikle, A. 2013. A polymorphism in the insulin-like growth factor 1 gene is associated with postpartum resumption of ovarian cyclicity in Holstein-Friesian cows under grazing conditions. Acta Vet. Scand. . 55: 11.

Othman, O. E., Abdel-Samad, M. F., El Maaty, N. A. A., Sewify, K. M. 2013. Genetic Characterization of Insulin Growth Factor-1 and Its Receptor Genes in Egyptian Buffalo (Bubalus bubalis L.). Br. Biotechnol. J. 3: 592.

Schoenau, W., Porciuncula, P., Zamberlan, G., Mesquita, F., Vieira, V., Oliveira, J., Gonçalves, P. 2005. Association between IGF-IR gene polymorphisms and productive and reproductive traits in Holstein cows. Arq. Bras. Med. Vet. Zootec. 57: 772-777.

Singh, U., Alyethodi, R., Alex, R., Kumar, S., Chakraborty, S., Dhama, K., Sharma, A., 2014 2014. Molecular markers and their applications in cattle

genetic research: A review. Biomarkers and Genomic Medicine 6:49-58.

Sosa, A., G., Kandiel, M., Eldebaky, H., Nawito, M., El-Roos, M. 2016. Genetic Polymorphism of Luteinizing Hormone Receptor Gene in Relation to Fertility of Egyptian Buffalo. Biotechnol. 12:

Zhou, H., Cheng, L., Azimu, W., Hodge, S., Edwards, G., Hickford, J. G. 2015. Variation in the bovine FABP4 gene affects milk yield and milk protein content in dairy cows. Sci. Rep. 5: 10023.